Properties of docetaxel-containing nanoemulsion as a dosage form

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Introduction

Efforts of scientists all over the world aimed at development of drug formulations that are biocompatible, cost-effective, non-irritating. Paclitaxel, the first of a new class of microtubule stabilizing agents, has been hailed by National Cancer Institute (NCI) as the most significant advance in chemotherapy of the past 15–20 years (Singla, 2002). Docetaxel belongs to the same class – taxanes, they are close in structure and properties. In many cases, especially for early stage cancer, undetectable cancer, metastatic cancer, or cancer that is not confined in solid tumor (e.g. leukemia) chemotherapy has been proved to be necessary and effective treatment.
Paclitaxel, one of the best antineoplastic drugs found from nature in the past decades, has been found effective against many forms of cancer, including ovarian cancer, breast cancer, small and non-small cell lung cancer, colon cancer, head and neck cancer, multiple myeloma, melanoma, and Kaposi's sarcoma (Feng, 2004). Unfortunately, most anticancer drugs have limitations in clinical administration due to their poor solubility and other unfavorable properties.
The approaches being used for a desired formulation of taxanes by various methods are co-solvency, emulsification, micellisation, liposome formation, non-liposomal lipid carriers (microspheres, nanocapsules etc.), cyclodextrins (CDs), local drug delivery devices and miscellaneous (Constantinides, 2004).
In present paper stabilized emulsions of oil-in-water type (o/w) with nano-scale particle sizes were used as containers for anticancer agents – taxanes (paclitaxel and docetaxel). The drug loaded lipid containing emulsion appeared to be one of the most convenient because of lasting storage, high drug solubilisation capacity and the prolongation of the drug release process. The aim of this study was to obtain nanoemulsion formulations and find out whether systems retain their properties and stability during storage.

Materials and methods

Commercially available cytostatic agent Docetaxel (Beijing Lunarsun Pharmaceutical Co., Ltd.), soybean lecithin Lipoid-S100 (Lipoid), bovine serum albumin BSA (Sigma), Soya oil from Glycine max (Fluka), 5% Dextrose, containing 0.026% NaCl and HCl (pH 3-4) and other reagents of special grade were used.

Preparation of the nanoemulsion

Crude emulsion containing docetaxel was formulated by stirring the water and oil phases when heating using a high-shear homogenizer. The stirring rate used for the manufacture was comprised between 10000 and 13000 rpm with a homogenizer Heidolph (Germany).
Further decrease of particle size was achieved by high pressure homogenization in APV (Germany) at 1800 bar for 4-5 times. The system was sterile filtered (0.22 mkm) for the preclinical studies.

The determination of the drug concentration
Docetaxel content in the emulsion was analyzed by high performance liquid chromatography (HPLC) on the analytical column Diaspher-110-C18 Biochemmack (Russia) (fig. 1). The mobile phase consisted of acetonitrile/water (80:20 (v/v)) at a flow rate of 0.5ml/min. A UV detector at 220 nm was used to detect and quantitate docetaxel. An aliquot was collected and diluted 1000 times by eluent before the analyzing. For standardisation, ethanol solutions of docetaxel were used (fig. 2)

Encapsulation efficiency measuring
To find out the drug loading capacity, probes were centrifuged at 1500 - 10000 rpm using Eppendorf Minispin (Germany). Besides it allowed to value stability of the system. The probe was diluted 5 times with water and centrifuged for 5 min. Drug content was monitored by HPLC.

The determination of the size of the internal phase of the emulsion
The mean particle size of the dosage form was determined by a dynamic light scattering method (DLS) using ALV-CGS-5022F (Germany). To take measurements, samples were diluted hundred times with sterile filtered water.

Stability examination
Aliquots of the emulsion were stored for two months at 4, 25 and 37°C and assayed for a physical and chemical stability. The mean particle size (DLS) and discoloration were used as indicators of physical stability. Once a week aliquots were picked out to assaying. Drug content was monitored during the stability program by HPLC.

Stability examination of the system diluted with dextrose infusion solution
The dosage form requires 10- or 20-fold dilution before use. After shaking, the system was diluted with 5% dextrose infusion solution. Stability studies of the emulsion were carried out at room temperature. Aliquots were picked out to assaying by HPLC. Before analyses, it was diluted 100, 50 times respectively by 0.1% TFA-containing eluent.

Modelling the drug release *in vitro*
The emulsion was diluted twenty times with water and *in vitro* release of docetaxel from oil droplets was monitored by membrane dialysis at 37 °C. A cellulose tube with a MW cut-off of 6-8 kDa was used. The sink solution was a 20мM phosphate buffer containing 1 mg/ml BSA. Concentrations of the drug in pre-/post-dialysis samples and aliquots at various time intervals were determined by HPLC and drug release profiles were generated.

The determination of the lecithin oxidability index
The sample of lipid-containing nanoemulsion was diluted 1000-2000 times by ethyl alcohol. Absorption spectrum at 400 – 200 nm was registered by UV-visible Recording Spectrophotometer Shimadzu UV-265FW (Japan). Ratios of absorbance at 270 and 215nm were compared. It is normal when <0.3.

Preclinical studies
To estimate whether the placebo possess a systemic toxicity 15 mice (BaLb/c, 20-22g) were divided into three groups. For the docetaxel-containing nanoemulsion 30 mice were divided into three groups (0.15, 0.3, 0.5 mg/mouse). The same experiment was held for traditionally used dosage form. The formulation was injected intraperitoneal. The emulsion was diluted 1:10 and 1:20 with a dextrose infusion solution.

Results and Discussion

The aim of our investigation was to obtain a new formulation with required characteristics, including high drug loading capacity, lasting storage, ease of use and possibility for the 24 hour continuous infusion.

Stable docetaxel-containing nanoemulsion was obtained. HPLC data show that docetaxel retention time is the same in emulsion (fig. 1) or in ethanol (fig. 2) solutions, and emulsion components go separately from the drug. The particle size of the final emulsion was small enough to sterilize it by filtration 0.22 mkm. The index of lecithin oxidability was normal.

The drug loading capacity is considered being 100%. Results of the docetaxel concentration determination after emulsion centrifugation are presented in table 1. The system did not show any phase separation or precipitation after centrifugation at 1500 - 3000 rpm.

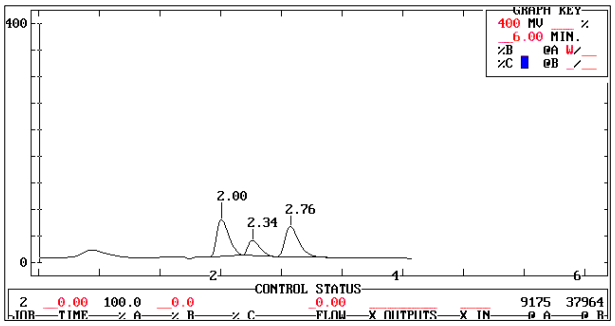

Fig. 1. Docetaxel-containing nanoemulsion; HPLC analysis

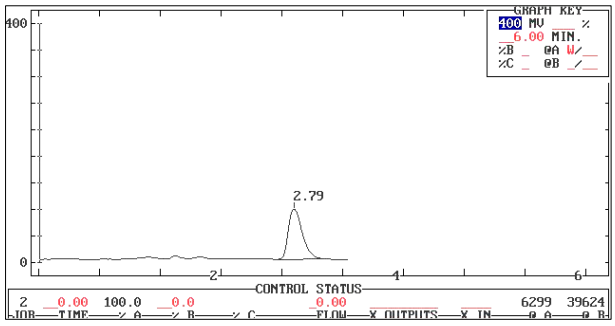

Fig. 2. Docetaxel-containing ethyl alcohol solution

According to the required techniques, the drug formulation was diluted in a ratio of 1:10 and 1:20 with saline dextrose infusion solution. Data presented in fig. 3 demonstrate that the system is absolute stabile for a day or two. In three days, 70% of the drug was still dissolved. To examine the system stability we used the method of accelerated ageing. The nanoemulsion was stored for two months at 37°C. The dynamic light scattering method proved that aggregation and precipitation did not happen (fig. 4). It is generally known, the system stability depends on particle sizes of the internal phase. The mean particle size of the studied formulation was bellow 200-300nm.

Conditions		Docetaxel content, %
rpm	min	
Original sample		100%
1500	5	98%
3000	5	98%
6000	5	89%
10000	5	48%

Table 1. Docetaxel content in the emulsion after centrifugation

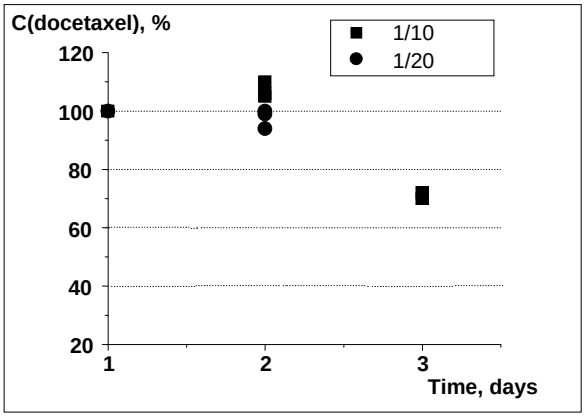

Fig. 3. Concentration of the drug in infusion solutions of emulsion (HPLC)

The formulation provides more gradual drug release in comparison with alcohol solutions used traditionally. This fact is illustrated in fig. 5. According to the results of dialysis, the drug release from the emulsion was slowed down (only 35% was found in sink solution after 1 day of incubation). As seen in fig. 5 (black dots), 100% of docetaxel was releasing from ethanol solution in 1 hour.

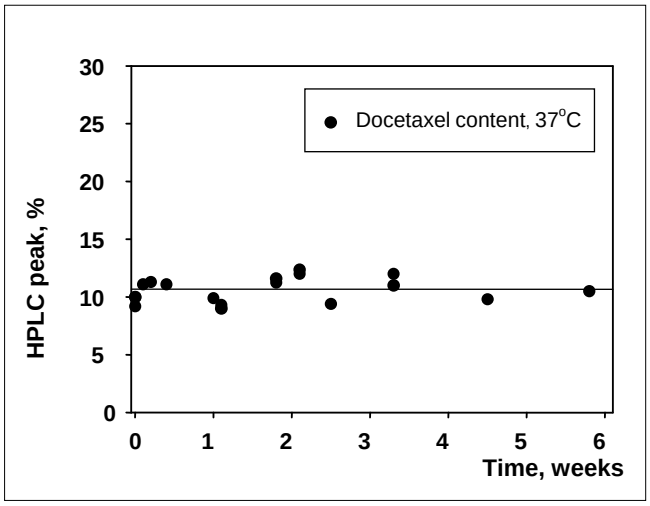

Fig. 4. Docetaxel content in emulsion during accelerated ageing at 37^{0}C

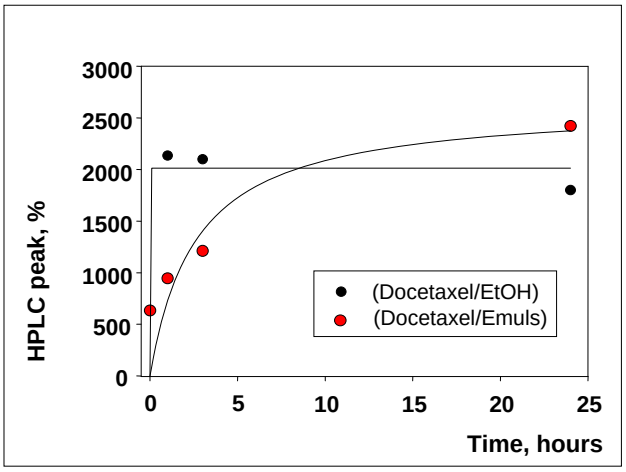

Fig. 5. Dialysis through a semi-permeable membrane against BSA-containing saline, blood plasma modelling

The placebo and docetaxel-containing dosage form successfully passed general toxicity test for mice. After placebo injection behaviour modification was not observed during seven days. Docetaxel-containing emulsion was analysed the same way. No mortality at maximum dose of 0.5 mg/mouse observed. The same dose of traditionally used formulation led to a death of three mice. At the moment trials with cancer cells are carried out.

Conclusions

In this study we showed that the lipid-containing emulsion of the o/w type could be used for docetaxel solubilisation. Nanoparticles of desired nano size and narrow size distribution were obtained. Docetaxel loading capacity considered to be 100% because no separation and concentration decrease were observed during the centrifugation and accelerated ageing. The dosage form is convenient for parenteral infusion during 24 hour. The concentration of taxane in samples remains constant after ten- / twentyfold dilution with saline infusion solution of 5% dextrose for a day. Dialysis, modelling drug circulation in blood plasma, demonstrates that nanoemulsion developed is stable for a long time preventing active compound from its fast release. We consider the lipid containing nanoemulsion to be a promising medicinal form.

References

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